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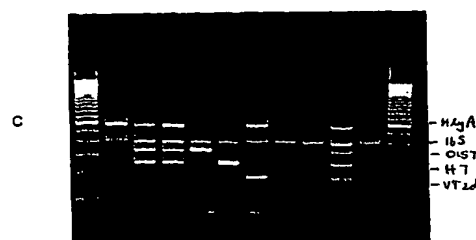
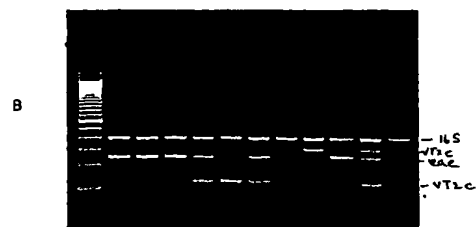
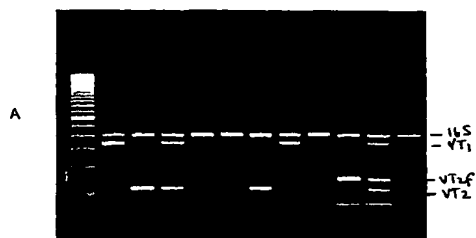
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- (81) Designated States (national): **AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.**
- (84) Designated States (regional): **ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),**

[Continued on next page]

(54) Title: **MAJOR VIRULENCE FACTOR DETECTION AND VEROCYTOTOXIN TYPE 2 SUBTYPE FROM CLINICAL *E. COLI* ISOLATES USING A ONE-STEP MULTIPLEX PCR**



(57) Abstract: A single kit comprising 3 multiplex PCR assays that can detect in *E. coli* the presence of the 8 virulence genes: *eaeA*, *EHEC-HlyA*, *Stx1* (VT1), *Stx2* (VT2), *Stx2c* (VT2c), *Stx2d* (VT2d), *Stx2e* (VT2e) and *Stx2f* (VT2f) is described. In addition, the kit can detect the two critical serotypes (O157 and H7) and identify the species (*Escherichia coli*) simultaneously using a one step reaction. Following evaluation in our hands, this PCR kit has been used to detect the above 11 components of disease-causing *E. coli* in a fast, accurate, reliable and specific fashion. These kits can be used on bacterial isolates and has the potential for use directly on foods and environmental samples.

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Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,  
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI,  
SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN,  
GQ, GW, ML, MR, NE, SN, TD, TG).

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**Published:**

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- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/FR 03/00042

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, MEDLINE, BIOSIS, Sequence Search, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PASS M A ET AL: "Multiplex PCRs for identification of Escherichia coli virulence genes." JOURNAL OF CLINICAL MICROBIOLOGY. UNITED STATES MAY 2000, vol. 38, no. 5, May 2000 (2000-05), pages 2001-2004, XP002252910 ISSN: 0095-1137 cited in the application abstract; figures 2-5 ----- -/--	1-3

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the International filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*G\* document member of the same patent family

Date of the actual completion of the international search

5 September 2003

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09.01.04

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 03/00042

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GANNON V P J ET AL: "USE OF THE FLAGELLAR H7 GENE AS A TARGET IN MULTIPLEX PCR ASSAYS AND IMPROVED SPECIFICITY IN IDENTIFICATION OF ENTEROHEMORRHAGIC ESCHERICHIA COLI STRAINS" JOURNAL OF CLINICAL MICROBIOLOGY, WASHINGTON, DC, US, vol. 35, no. 3, May 1997 (1997-05), pages 656-662, XP000957649 ISSN: 0095-1137 cited in the application abstract; figure 4; table 2	1-3
X	HU Y ET AL: "Rapid and sensitive detection of Escherichia coli 0157:H7 in bovine faeces by a multiplex PCR." JOURNAL OF APPLIED MICROBIOLOGY, vol. 87, no. 6, December 1999 (1999-12), pages 867-876, XP002252911 ISSN: 1364-5072 cited in the application abstract; figure 1; table 2	1-3
X	EP 0 669 399 A (SHIMADZU CORP) 30 August 1995 (1995-08-30) the whole document see in particular SEQ ID NO:2 and SEQ ID NO:13	1-3
A	US 5 652 102 A (WIEDMANN MARTIN ET AL) 29 July 1997 (1997-07-29) cited in the application the whole document	1-3
P,X	WANG, GEHUA ET AL: "Detection in Escherichia coli of the genes encoding the major virulence factors, the genes defining the 0157:H7 serotype, and components of the type 2 Shiga toxin family by multiplex PCR" JOURNAL OF CLINICAL MICROBIOLOGY (2002), 40(10), 3613-3619, XP002252912 the whole document	1-3
E	WO 03/052143 A (SIREVAAG REIDUN ;ANGLES D AURIAC MARC B (NO)) 26 June 2003 (2003-06-26) page 17 - page 21; tables 4,5	1-3

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/CA 03/00042

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see PCT/ISA/210

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-3 (all partially)

Primers specific for the E. coli virulence gene VT1, which primers consist of at least 15 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:2, kit comprising said primers and method using said primers.

---

2. claims: 1-3 (all partially)

Primers specific for the E. coli virulence gene VT2f, which primers consist of at least 15 contiguous nucleotides of SEQ ID NO:3 or SEQ ID NO:4, kit comprising said primers and method using said primers.

---

3. claims: 1-3 (all partially)

Primers specific for the E. coli virulence gene VT2, which primers consist of at least 15 contiguous nucleotides of SEQ ID NO:5 or SEQ ID NO:6, kit comprising said primers and method using said primers.

---

4. claims: 1-3 (all partially)

Primers specific for the E. coli virulence gene VT2c, which primers consist of at least 15 contiguous nucleotides of SEQ ID NO:7 or SEQ ID NO:8, kit comprising said primers and method using said primers.

---

5. claims: 1-3 (all partially)

Primers specific for the E. coli virulence gene VT2e, which primers consist of at least 15 contiguous nucleotides of SEQ ID NO:9 or SEQ ID NO:10, kit comprising said primers and method using said primers.

---

6. claims: 1-3 (all partially)

Primers specific for the E. coli virulence gene eaeA, which primers consist of at least 15 contiguous nucleotides of SEQ ID NO:11 or SEQ ID NO:12, kit comprising said primers and method using said primers.

---

7. claims: 1-3 (all partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Primers specific for the E. coli virulence gene VT2d, which primers consist of at least 15 contiguous nucleotides of SEQ ID NO:13 or SEQ ID NO:14, kit comprising said primers and method using said primers.  
---

8. claims: 1-3 (all partially)

Primers specific for the E. coli virulence gene HlyA, which primers consist of at least 15 contiguous nucleotides of SEQ ID NO:15 or SEQ ID NO:16, kit comprising said primers and method using said primers.  
---

9. claims: 1-3 (all partially)

Primers specific for the E. coli virulence gene rfbE, which primers consist of at least 15 contiguous nucleotides of SEQ ID NO:17 or SEQ ID NO:18, kit comprising said primers and method using said primers.  
---

10. claims: 1-3 (all partially)

Primers specific for the E. coli virulence gene fliC, which primers consist of at least 15 contiguous nucleotides of SEQ ID NO:19 or SEQ ID NO:20, kit comprising said primers and method using said primers.  
---

11. claims: 1-3 (all partially)

Primers specific for the E. coli 16S rRNA, which primers consist of at least 15 contiguous nucleotides of SEQ ID NO:21 or SEQ ID NO:22, kit comprising said primers and method using said primers.  
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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/JP 03/00042

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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			EP 0669399 A2	30-08-1995
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			US 5795717 A	18-08-1998
			US 6218110 B1	17-04-2001
US 5652102	A	29-07-1997	NONE	
WO 03052143	A	26-06-2003	WO 03052143 A2	26-06-2003